Taxonomy of *Juniperus deppeana* varieties and formas based on nrDNA (ITS), petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF sequences

Robert P. Adams
Biology Department, Baylor University, Box 97388, Waco, TX 76798, USA
Robert_Adams@baylor.edu

Andrea E. Schwarzbach
Department of Biomedicine, University of Texas at Brownsville, Brownsville, TX 78520, USA.

ABSTRACT

Juniperus deppeana has numerous disjunct populations that include four taxonomic varieties and three forms. All four varieties and two forms of *Juniperus deppeana* from the southwest United States, Mexico and Guatemala were analyzed by sequencing nrDNA (ITS), petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF. A Bayesian tree gave support for clades of var. *deppeana*, NM, var. *patoniana*, and var. *gamboana*. However, several clades with high support contain mixtures of different varieties and forms. A minimum spanning network based on 91 mutational events (MEs) showed that the varieties and forms are extremely closely related, differing by only 1 to 2 bp (out of 4411 bp). The taxon with the largest diffentiation was var. *deppeana*, Sacramento Mtns., NM that differed by 4 MEs from the Oak Creek canyon, AZ individuals. The lack of variation among *J. deppeana* taxa may be due to the mixing of populations during the Wisconsin glacial maximum (70,000 - 13,000 ybp) when life zones descended about 800 m. Published on-line: **www.phytologia.org** *Phytologia 95(2): 161-166 (May 1, 2013).*

KEY WORDS: *Juniperus deppeana* varieties, *Cupressaceae*, DNA, nrDNA (ITS), petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF, systematics, geographic variation, taxonomy.

Juniperus deppeana Steudel has trunk bark that exfoliates in quadrangular plates, thus the common name 'alligator bark' juniper. *Juniperus deppeana* is part of the serrate leaf margined species of the western hemisphere (Adams, 2011) and is widely distributed in the southwestern US, Mexico and northern Guatemala (Fig. 1). Putative *Juniperus d.* f. *sperryi*, once known only from the type locality in the Davis Mtns., TX, has been found in Arizona and New Mexico (Fig. 1). However, the bark characters seem to be controlled by only a few genes tuhus furrowed bark may have arisen independently in western Mexico and the southwestern United States (e.g., *J. d.* var. *patoniana* and *J. d.* f. *sperryi*). Whether the furrowed bark trees of Mexico are related to *J. d.* f. *sperryi* is not well understood.

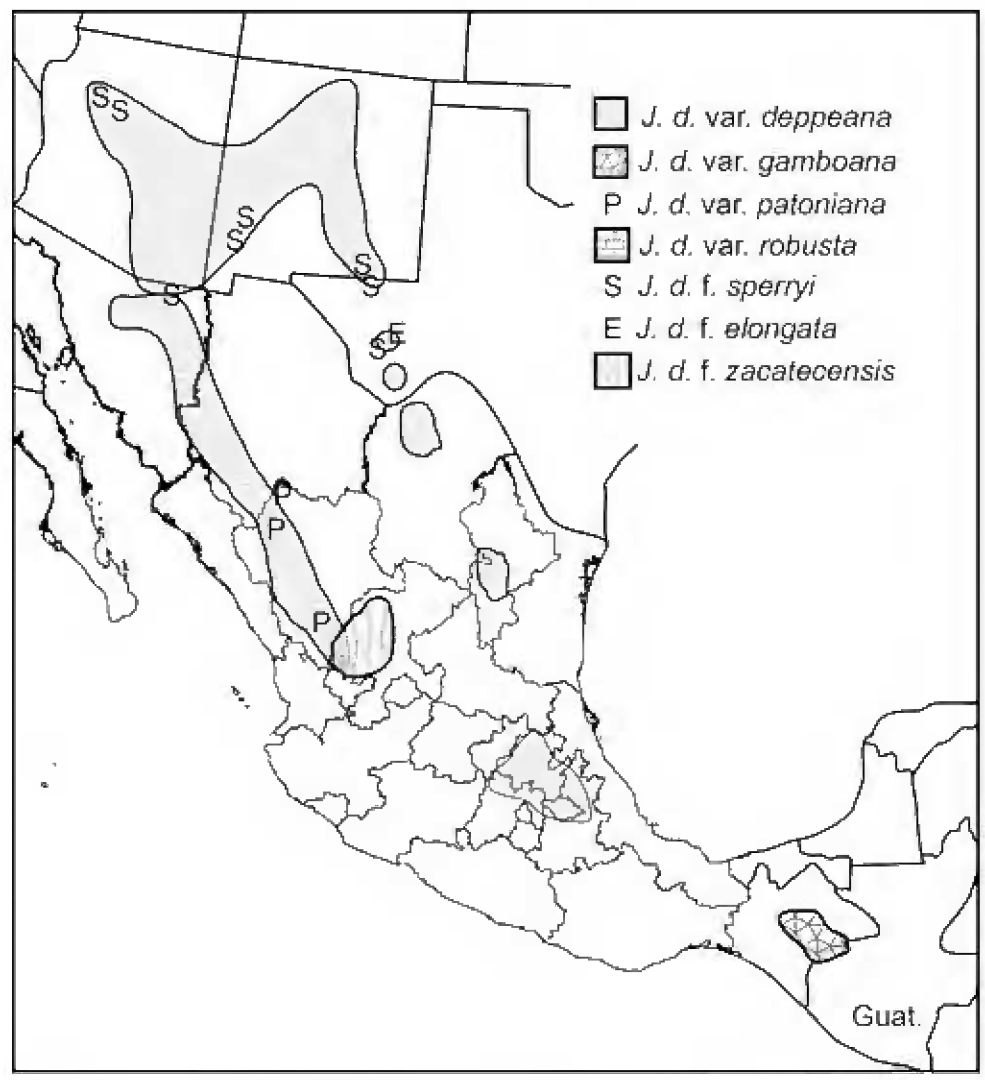

Figure 1. Distribution of *J. deppeana*.

The first systematic treatment of the serrate leaf-margined junipers was by Martinez (1963) who recognized *J. deppeana* Steudel. var. *deppeana* (checkered bark, (3)4-5(6) seeds/cone,

J. d. var. *pachyphlaea* (Torrey) Mart. (checkered bark, (1)2-4(5) seeds/cone), *J. d.* var. *robusta* Mart. (checkered bark, (1)2-3(-6) seeds/cone), *J. d.* var. *zacatecensis* Mart. (checkered bark, 1-4(-7) seeds/cone), *J. patoniana* Mart. (laced bark, (1)2-3(-6) seeds/cone, and *J. gamboana* Mart. (checkered bark, 1(2) seeds/cone) (Fig. 2).

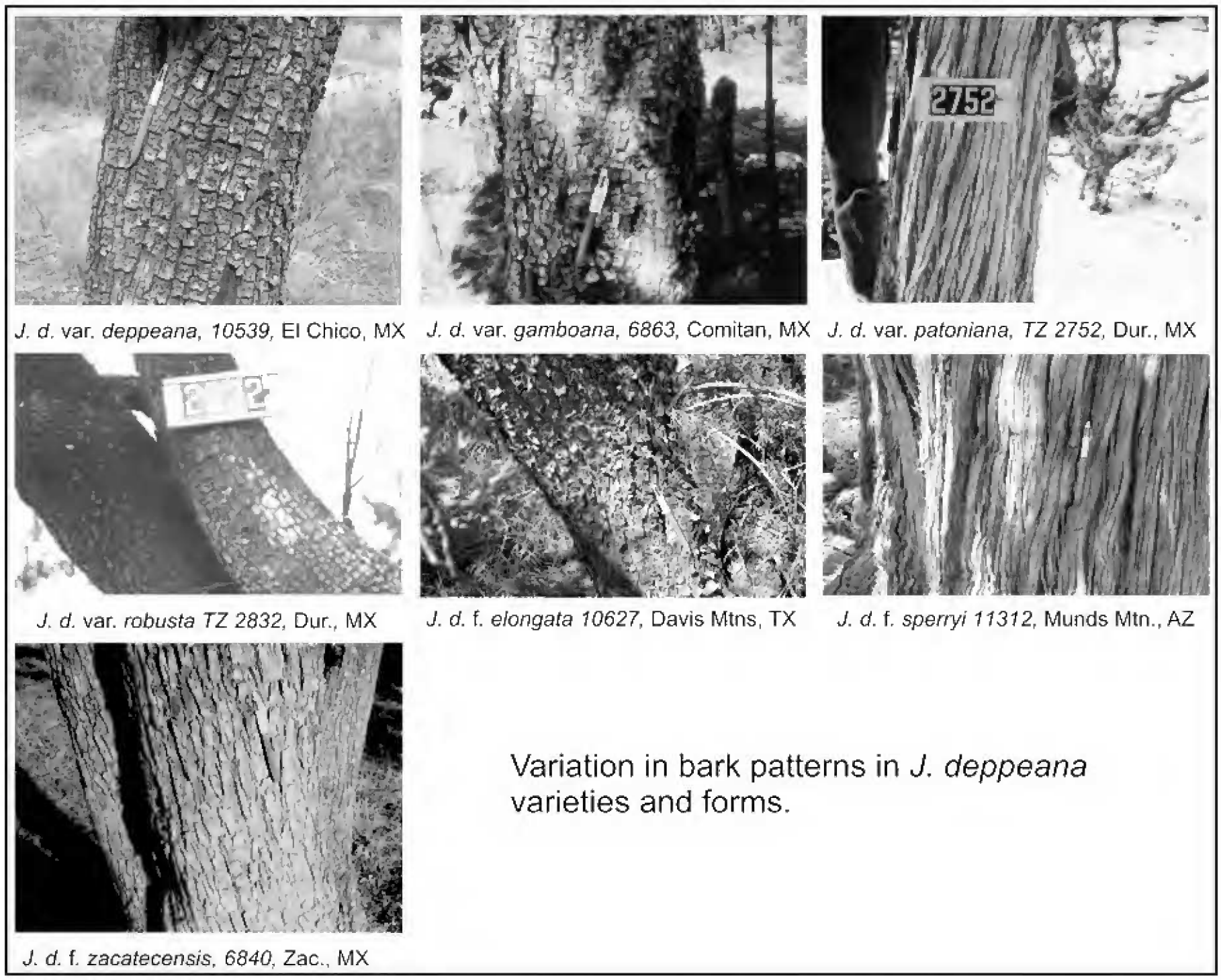

Figure 2. Variation in bark exfoliation among *J. deppeana* varieties and forms.

Zanoni and Adams (1976, 1979) and Adams, Zanoni and Hogge (1984), using morphology and essential oils, generally agreed with Martinez's treatment, except *J. patoniana* was reduced to *J. d.* var. *patoniana* (Mart.) Zanoni. Additional studies (Adams and Nguyen, 2005; Adams et al. 2007) have further clarified geographical variation in *J. deppeana*.

Recently, Adams and Schwarzbach (2006) recognized *J. gamboana* as *J. deppeana* var. *gamboana* (Mart.) R. P. Adams and *J. deppeana* var. *zacatecensis* as *J. deppeana* f. *zacatecensis* (Mart.) R. P. Adams. Adams and Schwarzbach (2011) found *J. deppeana* and var. *gamboana* to be a clade, sister to *J. ashei, J. saltillensis* and *J. zanonii* (Fig. 3). However, the other *J. deppeana* varieties and forms were not included in their study.

The focus of the present study was to examine relationships among all the recognized (Adams 2011) varieties and formas of *J. deppeana* (except the very minor variant f. *elongata*, Adams 2011) using data obtained from sequencing of nrDNA (ITS), petN-psbM, trnS-trnG, trnD-trnT and trnL-trnF.

MATERIALS AND METHODS

Specimens used in this study: *J. deppeana* var. *deppeana*, *Adams 10539-10541*, El Chico National Park, Hidalgo, MX; *Adams 7632-7634*, Sacramento Mtns., e of Alamogordo, NM, USA; Adams *10640-10642*, Oak Creek Canyon-Flagstaff, AZ; *J. deppeana* var. *gamboana*, *Adams 6863-6867*, Comitan, Chiapas, MX; *J. deppeana* var. *patoniana*, *Adams 6836-6839*, km 152, w. of Durango (city), Durango, MX (P); *J. deppeana* var. *robusta*, *Adams 10255-10256*, w of La Ciudad, Durango, MX; *J. deppeana* f. *sperryi*, *Adams 10626*, Bridge Spring, Davis Mtns., TX, USA; *Adams 11312*, Munds Mtn., AZ; *J. deppeana* f. *zacatecensis*, *Adams 6840-6842*, 18 km w. Sombrette, Zacatecas, MX; *J. virginiana*, *Adams 10231-10232*, Knoxville, TN, USA. Voucher specimens are deposited at BAYLU herbarium, Baylor University.

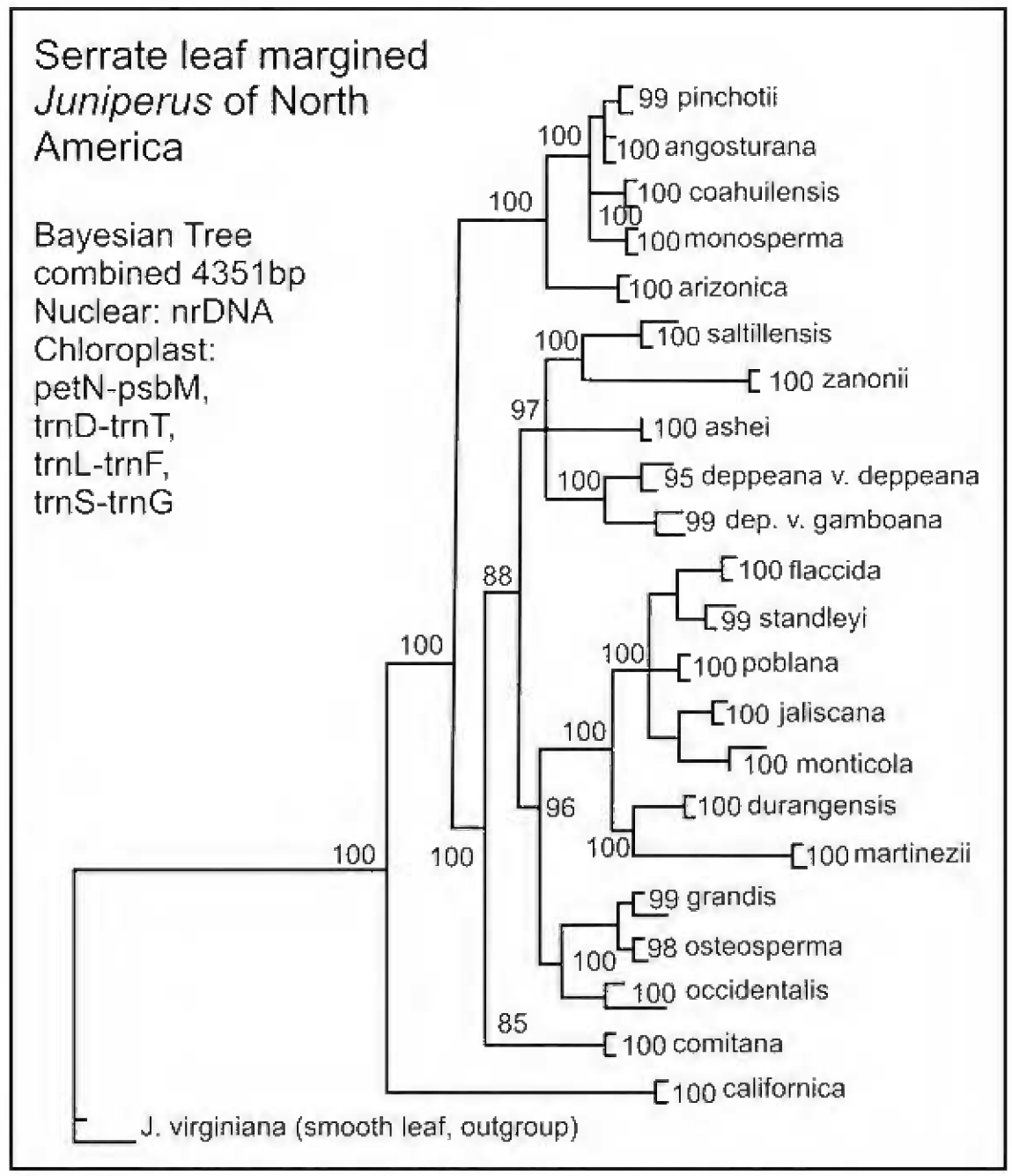

Figure 3. Bayesian tree of the serrate *Juniperus* of North America. Numbers at the branch points are posterior probabilities (as percent).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN, trnD-T, trnL-F, trnS-G) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized. The primers for trnD-trnT, trnL-trnF and trnS-trnG regions have been previously reported (Adams and Kauffmann, 2010).

The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.) or Sequencher v. 5 (genecodes.com). Sequence datasets were analyzed using Geneious v. 5.4 (Drummond et al. 2011), the MAFFT alignment program and the PAUP* program, version 4.0b10 (Swofford 2003) for neighbor joining, parsimony, and maximum likelihood tree searches. Further analyses utilized the Bayesian analysis software Mr. Bayes v.3.1

(Ronquist and Huelsenbeck 2003). For phylogenetic analyses, appropriate nucleotide substitution models were selected using Modeltest v3.7 (Posada and Crandall 1998) and Akaike's information criterion. Minimum spanning networks were constructed from mutational events (ME) data using PCODNA software (Adams et al., 2009; Adams, 1975; Veldman, 1967).

RESULTS AND DISCUSSION

Sequencing the five gene regions (nrDNA (ITS), petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF) resulted in 4411 bp of data. A Bayesian tree based on these data (Fig. 4) supports clades of var. *deppeana*, NM, var. *patoniana*, and var. *gamboana*. However, several clades with high support contain mixtures of different varieties and forms. Note that accessions of var. *robusta* are in different clades, as are the samples of f. *sperryi* from AZ and TX. It should be noted that hybridization between varieties and forms should be expected as these dioecious taxa are out-crossing plants in populations where several infraspecific taxa are often present.
the

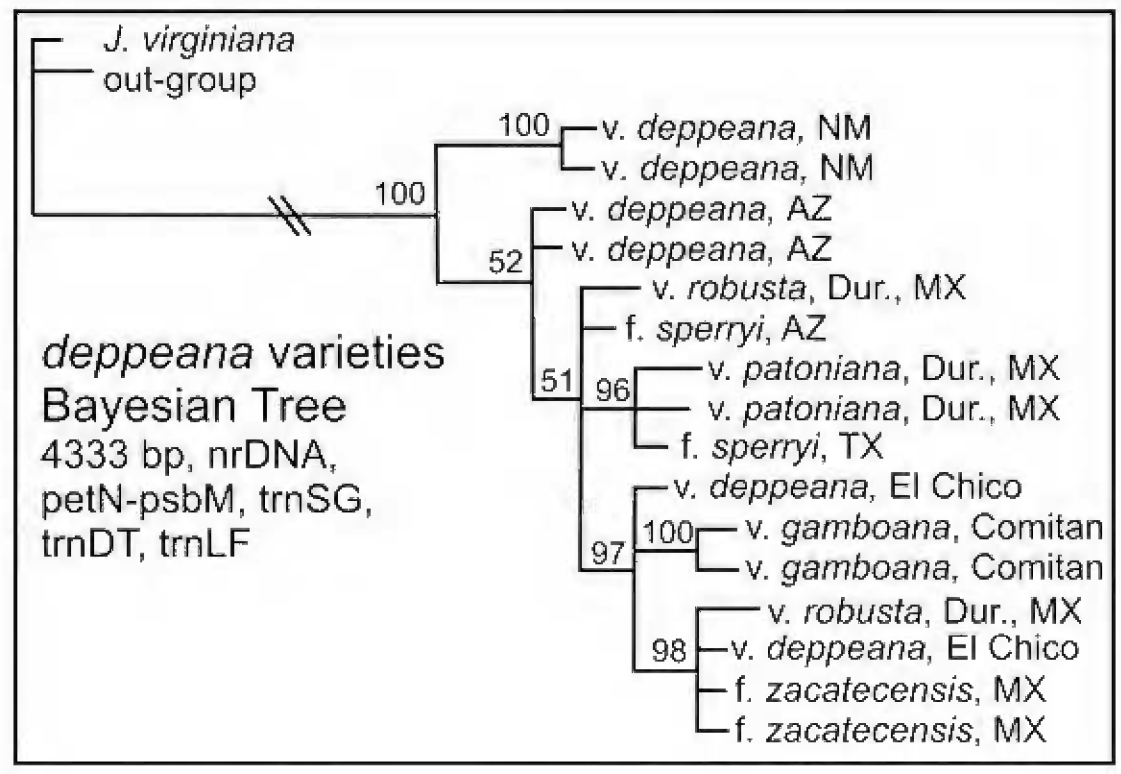

Figure 4. Bayesian tree of *J. deppeana* varieties. Numbers at branch points are posterior probabilities (as percent).

Although the Bayesian tree indicates high support for some clades (Fig. 4), the magnitude of differentiation among accessions is not apparent. A minimum spanning network based on all mutational events (MEs) assimilates both the nucleotide substitution and indel information. This analysis revealed 97 MEs, with 6 MEs found only once, and 91 MEs found multiple times. Of the 91 MEs, 70 differentiated *J. virginiana* from *J. deppeana* (Fig. 5). Thus, the entire differences among these 4 varieties and 2 forms amount to only 21 MEs. In general, only 1 or 2 MEs separate individuals (Fig. 5). The exception is the differentiation of plants from NM (Fig. 4) that are separated from the AZ plants by 4 MEs.

Adams and Schwarzbach (2012) found that traditionally recognized taxonomic species differed by 8 to 12 (or more) MEs, whereas varieties appeared to differ by less than 8 MEs. The differences between var.*deppeana* from AZ and NM, although interesting, do not appear to be

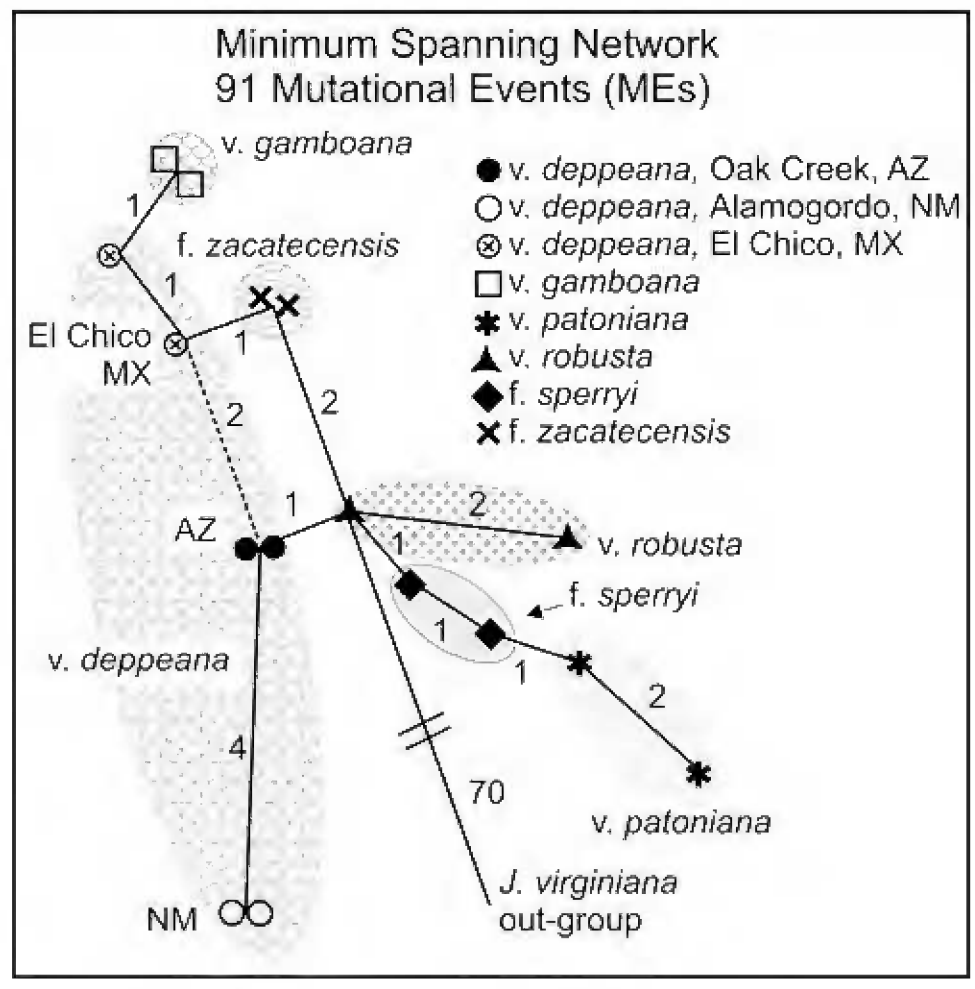

Figure 4. Minimum spanning network. Numbers on the lines are the number of MEs.

sufficient, by themselves, to warrant the recognition of a new variety from NM. Additional research on geographic variation in leaf terpenes and morphology is in progress so as to examine differentiation in the southwestern US.

CONCLUSION

The mixtures of various taxa within clades may be due to ancient climate and past distributions of *J. deppeana*. Wells (1966), using data from rat middens from the Big Bend of Trans-Pecos, Texas, concluded that during the Wisconsin (70,000 - 13,000 ybp) life zones descended about 800 m leading to the formation of a pinyon-juniper woodland in the present Chihuahuan desert between the Big Bend of Trans-Pecos, Texas and the city of Del Rio. Assuming that the effects of glaciation were mediated southward into Mexico so that life zones descended only a few hundred meters in Hidalgo, it appears that most of the now disjunct populations of *J. deppeana* may have once been connected in a nearly continuous population of distribution around the Chihuahuan desert (Fig. 5). It is likely that desert peaks within the area concerned also supported stands of *J. deppeana*. Wisconsin populations would have become spatially separated as dryer, warmer climate developed during the Holocene (past 13,000 y). Of course, the Wisconsin was only the most recent of several pluvial events during the Pleistocene, spanning 1.8 my (Flint, 1971). It is likely that during any one (or several) of these pluvial events, *Juniperus deppeana* occupied lower elevation and more southward habitats, leading to more contiguous populations in Mexico and the southwestern United States. If divergent populations (or varieties) became sympatric during the Wisconsin, this would have facilitated infra-specific crossing. This may account for the large genetic variation within some populations. In addition, the millennia of continuous populations could explain the lack of differentiation between the recently (Holocene) geographically isolated populations.

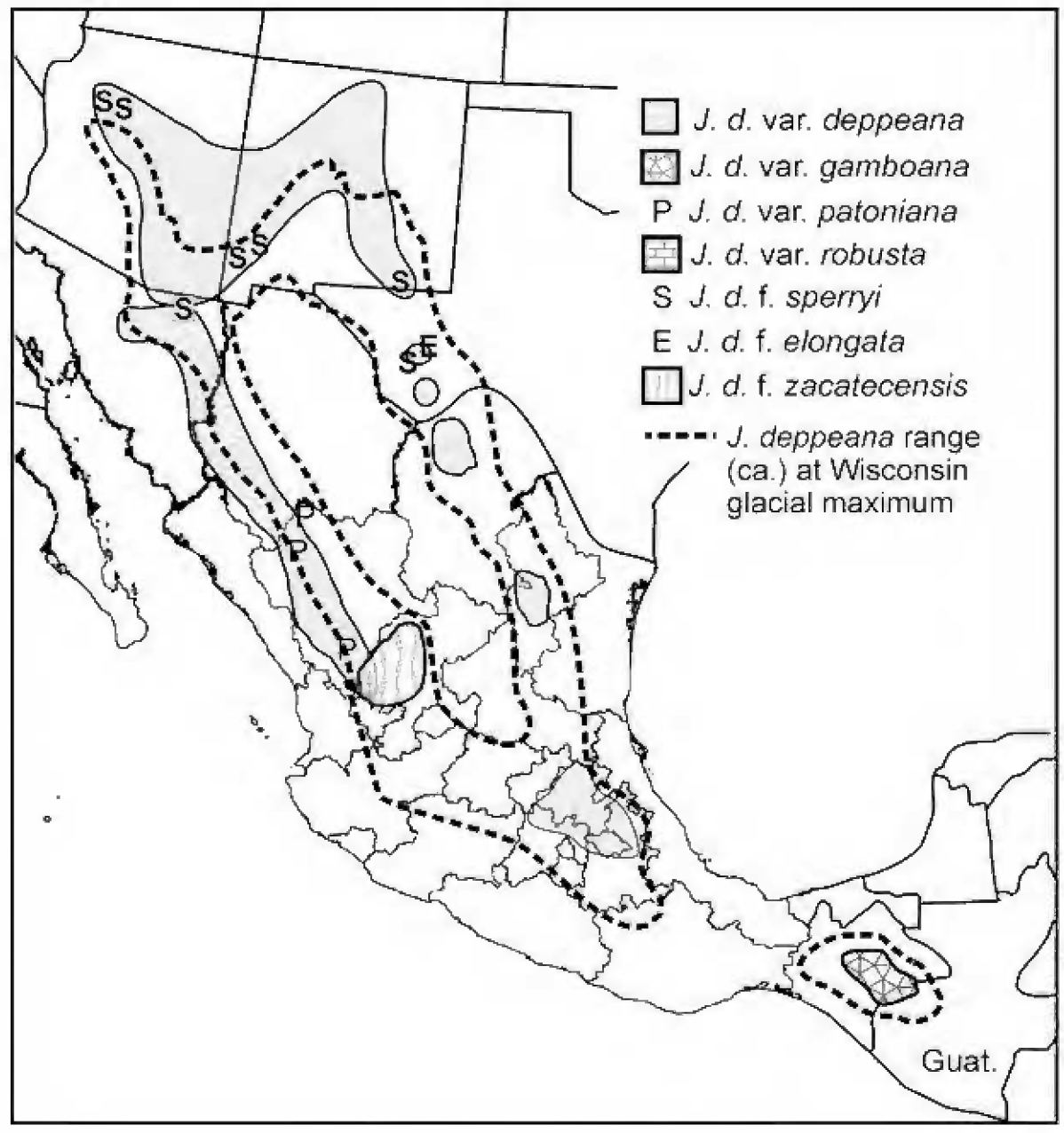

Figure 5. Possible range of *J. deppeana* during the Wisconsin glacial maximum (based on Wells, 1966). The present day disjunct populations were likely continuous in the foothills around the Chihuahuan desert during the Wisconsin.

ACKNOWLEDGEMENTS

Thanks to Tom Zanoni (NYBG) for providing slides of tree barks and assistance in field work, and Tonnie Yanke for lab assistance. This research was supported in part with funds from NSF grant DEB-316686 (A. Schwarzbach and R. P. Adams) and funds from Baylor University.

LITERATURE CITED

Adams, R. P. 1975. Statistical character weighting and similarity stability. Brittonia 27: 305-316.

Adams, R. P. 2011. The junipers of the world: The genus *Juniperus*. 3rd ed. Trafford Publ., Victoria, BC.

Adams, R. P., J. A. Bartel and R. A. Price. 2009. A new genus, *Hesperocyparis*, for the cypresses of the new world. Phytologia 91: 160-185.

Adams, R. P. and S. Nguyen. 2005. Infra-specific variation in *Juniperus deppeana* and f. *sperryi* in the Davis Mountains, Texas: Variation in leaf essential oils and Random Amplified Polymorphic DNAs (RAPDs). Phytologia 87:96-108.

Adams, R. P. and A. E. Schwarzbach. 2006. Infraspecific adjustments in *Juniperus deppeana* (Cupressaceae) Phytologia 88: 227-232.

Adams, R. P. and A. E. Schwarzbach. 2011. DNA barcoding a juniper: the case of the south Texas Duval county juniper and serrate junipers of North America. Phytologia : 146-154.

Adams, R. P. and A. E. Schwarzbach. 2012. Taxonomy of the multi-seeded, entire leaf taxa of *Juniperus* section *Sabina*: Sequence analysis of nrDNA and four cpDNA regions. Phytologia 94: 350-368.

Adams, R. P., T. A. Zanoni and L. Hogge. 1984 Analyses of the volatile oils of *Juniperus deppeana* and its infraspecific taxa: chemosystematic implications. Biochem. Syst. and Ecol. 12:23-28.

Adams, R. P., A. E. Schwarzbach, S. Nguyen, and J. A. Morris. 2007. Geographic variation in *Juniperus deppeana.* Phytologia 89: 132-150.

Axelrod, D. I. 1958. Evolution of the Madro-Tertiary geoflora. Bot.Rev. 24: 433- 509.

Drummond A. J., B. Ashton, S. Buxton, M. Cheung, A. Cooper, C. Duran, M. Field, J. Heled , M. Kearse, S. Markowitz, R. Moir, S. Stones-Havas, S. Sturrock, T. Thierer and A. Wilson. 2011. Geneious v 5.4, Available from http://www.geneious.com/

Flint, R. F. 1971. Glacial and quaternary geology. John Wiley and Sons, NY.

Martinez, M. 1963. Las pinaceas mexicanas. Tercera edicion. Universidad Nacional Autonoma de Mexico. Mexico City.

Posada, D. and K. A. Crandall. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14: 817-818.

Ronquist, F. and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572-1574.

Swofford, D. L. 2003. PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer Associates, Sunderland, MA.

Veldman, D. J. 1967. Fortran programming for the behavioral sciences. Holt, Rinehart and Winston Publ., NY.

Wells, P. V. 1966. Late Pleistocene vegetation and degree of pluvial climatic change in the Chihuahuan desert. Science 153: 970-975.

Zanoni, T. A. and R. P. Adams. 1976. The genus *Juniperus* (Cupressaceae) in Mexico and Guatemala: Numerical and chemosystematic analysis. Biochem. Syst. Ecol. 4: 147-158.

Zanoni, T. A. and R. P. Adams. 1979. The genus *Juniperus* (Cupressaceae) in Mexico and Guatemala: Synonymy, Key, and distributions of the taxa. Bol. Soc. Bot. Mexico 39: 83-121.